Vol. XXXIV. *June, 1918.* *No. 6.*

BIOLOGICAL BULLETIN

EFFECT OF ENVIRONMENT UPON INHERITED CHARACTERS OF *HYDATINA SENTA*.

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INTRODUCTION.

Several years ago it was discovered (Shull, 1915) that two distinct parthenogenetic lines of the rotifer *Hydatina senta*, one from England, the other from Nebraska, differed in certain physiological (and perhaps structural) characters: (1) the English line laid smaller eggs than the Nebraska line; (2) the English line habitually laid a large percentage of its eggs attached to the surface film of the water, while the Nebraska line laid most of its eggs at the bottom or sides of the vessel; (3) the eggs of the English line required longer to develop than did those of the Nebraska line; (4) and when the rotifers were killed in Bouin's fluid the foot was seldom, or only slightly, retracted in the English line, but considerably retracted in the Nebraska line.

When crosses were effected between these two lines, the F_1 lines and F_2 lines were all indistinguishable from the English line in all the above-named characteristics. It seemed as if segregation and recombination had failed, and that in some way the four characters were rigidly associated one with another.

At first it was regarded as possible that the four characters were not really distinct, but were different manifestations of a single (physiological) character. That character might have been a greater permeability of the cells in one line than in the other. Thus, if the Nebraska line were more permeable to oxygen, the increased metabolism might make its eggs larger. For the same reason the eggs of the Nebraska line might develop in less time. In like manner it might be that the Nebraska rotifers, able to get the required amount of oxygen at the bottom

of the dish where the oxygen in solution was less abundant, lived most of the time at the bottom and laid their eggs there; whereas the less permeable English rotifers were forced to swim to the surface where dissolved oxygen was presumably more abundant, and laid eggs at the surface film. And if the foot muscles of the Nebraska line were more permeable to the killing fluid than were those of the English line, the greater contraction of the muscles of the former might thereby be explained.

It was possible to test the correctness of the above assumptions, in part, by artifically altering the expression of the inherited characters through changes in the environment. A number of experiments were performed to this end. However, before they were completed, an F_3 generation was obtained in which the association of the four inherited characters was broken. In this and the succeeding generations each one of the four characters was separated at least once from the others with which it was associated in the original lines.

Thus was proven that the four characters were not merely different expressions of one character. The experiments designed to test their separateness or singleness were, therefore, not completed, and were not published. It has now become necessary, however, to refer to certain of the results, and they are here described in the incomplete form in which they were left. Along with them are several experiments on the viability of fertilized eggs, as affected by external conditions. These are of interest to the experimenter from a practical standpoint, and also in relation to popular ideas concerning the fertilized eggs of *Cladocera*.

EXPERIMENTS.

Effect of oxygen upon the laying of eggs at surface and bottom of water.

On each of the dates named in Table I., approximately equal numbers of females of *Hydatina* were placed in two dishes. In one was placed water oxygenated by vigorously shaking it in an atmosphere having a high percentage of oxygen. The dish was then set, uncovered, under a bell jar in which was confined an atmosphere containing the same high percentage of oxygen as that with which the water was first saturated. Since the bell

jar was being used daily for other experiments, the percentage of oxygen used was not always the same. From May 27 to June 22, and on July 8 (see Table I.), the atmosphere contained 40 per cent. of oxygen; on all other dates 60 per cent.

In the other of the two dishes was placed untreated water. This dish was also set under a jar sealed at the edges, to prevent excess of evaporation, but in ordinary air.

After 20 to 40 hours the dishes were removed and the number of eggs at the surface film and at the bottom counted. As shown in Table I., the eggs are much less abundant at the surface film in the presence of much oxygen than in air.

TABLE I.

Showing the Number of Eggs Laid at the Surface Film and at the Bottom of the Dish by Rotifers Placed in Air and in an Atmosphere Containing an Excess of Oxygen.

Date.	Air.		Excess of Oxygen.	
	Number of Eggs at Surface.	Number of Eggs at Bottom.	Number of Eggs at Surface.	Number of Eggs at Bottom.
May 27	12	5	1	15
29	37	48	16	55
June 3	22	22	16	27
22	2	42	0	25
28	62	20	19	49
29	8	25	7	29
30	43	85	18	121
July 1	38	19	17	27
2	40	33	34	39
3	45	6	14	41
4	42	36	23	76
6	12	5	8	22
8	23	10	12	22
Total	386	356	185	548
Percentage at surface	52.0		25.2	

Effect of oxygen upon the size of parthenogenetic eggs.

Eggs laid by rotifers in oxygenated water, and in untreated water, were obtained in the following manner. Three or four young females, due to begin egg laying in 6 to 12 hours, were placed in each of two watch glasses. One lot was immersed in water saturated with an atmosphere of which 40 per cent. or 60 per cent. was oxygen, and then set under a bell jar in a similar atmosphere. The other lot was placed in spring water, and the dish was set under a closed bell jar in air. After 24 hours the

watch glasses were removed, and all the eggs that were in a horizontal position were measured by means of a screw micrometer eye-piece. The measurements are given in units of the scale. They are directly comparable with the measurement of eggs in my former paper (Shull, 1915) since all the measurements were made with the same microscope and with the same lenses.

The mean length, mean breadth, standard deviation, etc., of the two lots of eggs are given in Table II.

TABLE II.

A Comparison of the Eggs Laid by the Rotifer Hydatina Senta in Oxygenated Water and Untreated Water.

	In Oxygenated Water.	In Untreated Water.	Difference.
Number of eggs measured......	100	100	0
Mean length of eggs...........	16.208 ± 0.028	16.098 ± 0.027	0.110 ± 0.039
Mean breadth of eggs.........	14.054 ± 0.021	14.021 ± 0.018	0.033 ± 0.028
Standard deviation of length...	0.409 ± 0.019	0.404 ± 0.019	0.005 ± 0.026
Standard deviation of breadth..	0.308 ± 0.015	0.266 ± 0.013	0.042 ± 0.019

The eggs in the oxygenated water were a trifle larger than those in the untreated water, though it can hardly be said that the difference is statistically significant. However, a difference that cannot be proven by statistical treatment to be significant is not necessarily insignificant. It seems to me not improbable that the difference in length is significant, but it is very small in comparison with the difference between English and Nebraska eggs described in my earlier publication (*op. cit.*).

Effect of oxygen upon the time of development of parthenogenetic eggs.

A number of egg-laying females were put into each of two dishes in the evening. In one dish was placed ordinary water, in the other water that had been oxygenated in the manner described in the preceding experiments. The females were removed after about an hour, but the eggs which they had laid were left in the dishes. The oxygenated water in one dish was then removed and replaced with fresh oxygenated water, and the dish was set under a bell jar in an atmosphere containing an excess of oxygen (40 or 60 per cent.). To insure that mechanical disturbance or

accumulation of metabolic products did not affect the time of development of the eggs unequally, the water in the control dish was also drawn off after the rotifers were removed and replaced with fresh water. This dish was then set under a bell jar in air.

The next morning the two dishes were examined at intervals of 20 to 30 minutes, and the young rotifers removed and counted as they hatched. In this way the approximate time of development of the eggs was determined. In Table III. these times are collected in groups, to the nearest half hour. When these figures are treated statistically, they compare with one another as in Table IV.

TABLE III.

Showing the Time Required for Development of Eggs in Oxygenated Water and in Untreated Water.

	Number of Eggs Hatching in (Hours).						
	11.5.	12 0.	12.5.	13.0.	13.5.	14.0.	14.5.
Oxygenated water...........	7	10	15	23	9	1	
Untreated water............	7	8	12	15	14	4	1

TABLE IV.

Showing the Time of Development of Eggs in Oxygenated Water and in Untreated Water. Statistical Treatment of Data in Table III.

	In Oxygenated Water.	In Untreated Water.	Difference.
Mean time of development in hours......................	12.654 ± 0.104	12.803 ± 0.129	0.149 ± 0.165
Standard deviation of time of development...............	1.239 ± 0.074	1.497 ± 0.091	0.258 ± 0.117

The eggs in oxygenated water hatched in a trifle shorter time, and somewhat more uniformly, though the difference in each case is so small that it may be insignificant. The greater uniformity of the time of development in oxygenated water (second line of Table IV.) is not improbably significant.

Effect of Oxygen upon the Contraction of the Foot Muscles.

Rotifers were placed in water saturated with an atmosphere containing 40 per cent. of oxygen, under a bell jar containing a similar atmosphere, and kept there 24 hours. They were then

removed and promptly killed in Bouin's fluid. Other rotifers from the same families were kept in ordinary water, under a bell jar in air, for 24 hours, then killed in Bouin's fluid.

The contraction of the foot muscles was noted in accordance with the following arbitrarily chosen degrees of contraction: (0) foot fully extended; (1) foot slightly contracted, toes bent to one side, but still visible; (2) foot considerably contracted, toes wholly concealed, but contraction limited to small region near toes; and (3) foot greatly contracted, region of contraction much greater than in preceding class. It is to be noted that this classification is not the same as that proposed for the English and Nebraska rotifers in my former paper (Shull, 1915). The descriptions there given were not applicable to the rotifers used in these experiments.

The degrees of contraction of the foot muscles of the two lots of rotifers is tabulated in Table V., and the statistical comparison of the figures in Table VI.

Table V.

Showing the Degree of Contraction of the Foot Muscles of Rotifers Kept in Oxygenated Water, and in Untreated Water, and Then Killed in Bouin's Fluid.

Degree of Foot Contraction.	Number of Rotifers of Given Foot Contraction.	
	In Oxygenated Water.	In Untreated Water.
0	35	21
1	97	111
2	64	64
3	2	2

Table VI.

Statistical Comparison of Data in Table V.

	In Oxygenated Water.	In Untreated Water.	Difference.
Mean foot contraction	1.16 ± 0.034	1.27 ± 0.030	0.11 ± 0.046
Standard deviation of foot contraction	0.64 ± 0.021	0.71 ± 0.024	0.07 ± 0.031

The statistical treatment is based on the assumption that each degree of contraction of the foot muscles differs from the degrees next to it by unity.

As shown in Table VI. there is less contraction of the foot

muscles in oxygenated water than in untreated water, but the difference is rather small compared with its probable error and in view of the crudity of the method of measurement may be without significance.

Effect of Temperature upon the Laying of Eggs at Surface and Bottom.

Equal numbers of rotifers were placed in a number of dishes of water. Some of the dishes were kept at room temperature which was fairly constant at 20° to 22° C. Other dishes were kept much cooler by setting them on a window sill outside. A thermometer was kept beside the latter dishes, and was fre-

TABLE VII.

The Number of Eggs Laid at the Surface Film and at the Bottom at Room Temperature and at Considerably Lower Temperatures.

Date.	Room Temperature.		Low Temperature.		
	Number of Eggs at Surface.	Number of Eggs at Bottom.	Number of Eggs at Surface.	Number of Eggs at Bottom.	Temperature in Degrees C.
Nov. 28.....	44	74	1	6	8
Nov. 29.....	20	59	0	14	7
			0	8	
			0	9	
			0	9	
Dec. 3.....	8	20	0	6	4
			0	10	
			0	4	
			0	2	
			0	0	
Dec. 4.....	15	22	0	0	2
			0	1	
			0	1	
			0	0	
Dec. 7.....	74	20	0	3	3
			0	1	
			0	1	
Dec. 8.....	6	20	3	7	12
			2	19	
			0	0	4
			0	3	
Totals......	167	215	6	104	
Percentage at surface....	43.7		5.5		

quently read. In Table VII., which gives the number of eggs laid at the surface film and at the bottom, the stated temperature

of these cool dishes is an estimated average. Because fewer eggs were to be expected at such low temperatures, several dishes were kept at low temperature for every one at room temperature.

There is no question here that the eggs are laid more largely at the surface when the temperature is high. The experiment was repeated on a small scale with the same result.

Effect of Temperature upon the Viability of the Fertilized Eggs.

Freezing.—Freezing of fertilized eggs was designed as a practical measure only, in order to induce those eggs to hatch which would not otherwise hatch. The attempt failed, however, as shown by the following tests.

In the first experiment about 244 eggs that remained unhatched after hatching had ceased for 12 days in the lot of eggs to which they belonged, were divided into two approximately equal groups. One group was frozen over night by immersing, in a closed vessel, in a brine-ice mixture and the other was kept in water at ordinary temperature. Of the frozen lot none hatched in 19 days thereafter. Of the control lot at room temperature, one hatched in 10 days after the beginning of the experiment, none thereafter. Freezing did not facilitate the hatching.

In the second experiment about 230 eggs that remained unhatched for 14 days after hatching of the eggs in the same lot had practically ceased were similarly divided into two groups, one of which was frozen over night and the other kept at room temperature. None of these eggs in either lot hatched in 19 days, after which time observation ceased.

From these experiments it appears that fertilized eggs not ordinarily capable of hatching can not be made to hatch by freezing.

Low Temperature Above Freezing.—Two lots of fertilized eggs from the same source and of approximately equal numbers were kept at different temperatures from the time they were laid until hatching was nearly complete. The eggs were laid between November 29 and December 8. One lot was kept at room temperature. The other was set outside on a window sill where daytime temperature, as shown by a thermometer placed beside

the dishes, ranged from 2° to 12° C. The air temperature was much below this, but was moderated in the location of the eggs by a steam radiator near the window sill inside. It appears that the water in which the eggs were placed never froze and hatching occurred during this period of low temperature. From December 22 to December 25 the atmospheric temperature was considerably above freezing but was much colder thereafter. On January 3 the "cold" dish was removed to room temperature, but hatching had been nearly completed before that time. Table VIII. shows the number of eggs that hatched.

TABLE VIII.

Showing the Effect of Room Temperature and Lower Temperatures Upon the Hatching of the Fertilized Eggs of Hydatina Senta.

Date of Beginning Experiment.	Warm.			Cold.		
	Number of Eggs.	Date of Hatching.	Number Hatched.	Number of Eggs.	Date of Hatching.	Number Hatched.
Nov. 29....	44	Dec. 6	1	43	Dec. 23	1
Dec. 2....	10	Dec. 7	1	10	Dec. 23	2
					Dec. 24	2
					Jan. 5	1
Dec. 8....	43	Dec. 10	2	44	Dec. 10	1
					Dec. 12	2
					Dec. 24	1
Total number hatching..........			4			10

Low temperature appears to favor the hatching of the eggs, not as an after effect, but during the period of low temperature.

Effect of Oxygen upon the Viability of the Fertilized Eggs.

Each of four lots of eggs from a single source was divided into two equal parts. One was placed in water of high oxygen content (saturated with an atmosphere of which either 40 per cent. or 60 per cent. was oxygen) and set under a bell jar enclosing an atmosphere containing the same proportion of oxygen as that with which the water was originally saturated. Atmospheres of 40 per cent. oxygen were used until July 7, 60 per cent. thereafter. This dish was removed from the bell jar daily and examined for hatching rotifers. The water was drawn off the eggs after examination and replaced with fresh oxygenated water and the dish returned to the bell jar.

The other dish was filled with untreated water which was drawn off daily and replaced with fresh water.

The hatching of the eggs is recorded in Table IX.

Table IX.

Showing the Effect of Oxygenation of the Water Upon the Hatching of the Fertilized Eggs of Hydatina Senta.

Experiment.	Date of Starting Experiment.	Oxygenated Water.			Untreated Water.		
		Number of Eggs.	Date.	Number Hatching.	Number of Eggs.	Date.	Number Hatching.
A.......	July 1, 1915	24	July 3	1	24	July 2	1
			7	1		7	1
			9	1		8	2
						12	1
				3			5
B.......	July 2, 1915	30	July 6	4	30	July 5	4
			10	1		8	1
			21	1		9	1
						10	1
						17	1
				6			8
C.......	July 4, 1915	79	July 5	1	78	July 8	1
			9	4		9	5
			10	5		10	4
			11	2		11	1
			12	3		12	3
						13	1
						14	1
				15			16
D.......	July 7, 1915	100	July 10	1	100	July 8	1
			11	2		9	1
			12	2		10	1
			12	5		11	3
			14	2		12	1
						13	8
						14	3
						21	1
						23	1
				12			20
Grand total.....	....			36	....		49

The eggs in oxygenated water show a somewhat lower viability in every case.

Effect of Drying upon the Viability of the Fertilized Eggs.

Dried for a Short Period.—A lot of cross-fertilized eggs—eggs laid by females of line B fertilized by males of line A of my former experiments (Shull, 1913)—were kept for seven weeks to allow hatching to take place. Practically all the hatching occurred in the second to fourth weeks of this period, almost none in the last three weeks. The 446 eggs (about two thirds of the original lot) which remained unhatched after seven weeks were divided into two nearly equal groups. One half was allowed to become dry December 8 and remain dry about 13 hours, after which it was remoistened. The other half was kept wet. The subsequent hatching of eggs from these two groups is recorded in Table X.

TABLE X.

Showing the Effect of Drying Eggs for a Short Period Upon the Proportion that Hatch in a Cross-Fertilized Lot of Eggs of Hydatina Senta.

Eggs Kept Wet.		Eggs Dried and Remoistened.	
Date.	Number Hatching.	Date.	Number Hatching.
Dec. 9	0		
10	0	Dec. 10	0
11	1	11	0
12	0	12	0
13	0	13	2
14	0	14	3
15	0	15	1
16	0	16	8
17	0	17	4
18	1	18	5
19	0	19	4
20	0	20	7
Total.	2		34

Observations necessarily stopped December 20, but it seems likely that even more would have hatched among the eggs that were dried. Drying for a short period either favors hatching of eggs that would not otherwise have hatched or hastens the hatching of eggs whose hatching would otherwise have been spread over a long period.

The above experiment was repeated with a lot of eggs from the reciprocal cross of the foregoing—eggs laid by females of line A fertilized by males of line B. The original lot of eggs was kept seven weeks to allow of hatching. During the last three weeks

of that time very little hatching occurred. The 238 eggs that remained (less than half of the original lot) were divided into two equal parts, one of which was allowed to become dry and remain so for 13 hours on December 4. The subsequent hatching is recorded in Table XI. The results confirm the conclusions drawn from Table X.

Table XI.

Showing the Effect of Drying Eggs for a Short Period Upon the Proportion that Hatch in a Cross-Fertilized Lot of Eggs of Hydatina Senta. The Cross was the Reciprocal of that in Table X.

Eggs Kept Wet.		Eggs Dried and Remoistened.	
Date.	Number Hatching.	Date.	Number Hatching.
Dec. 5–20	0	Dec. 7–15	0
		16	1
		17	1
		18	16
		19	2
		20	1
Total	0		21

In another experiment of this kind inbred eggs (eggs laid by females fertilized by males of the same line) of a line in which a relatively small proportion of the eggs normally hatched were used. The eggs were kept five weeks, during the last 12 days of which time no eggs hatched. The eggs that remained unhatched were divided into two lots which, by mistake, were made unequal. One lot, consisting of about 130 eggs, was dried over night; the other of 106 eggs, was kept wet. The subsequent hatching of these eggs is recorded in Table XII.

Table XII.

Showing the Effect of Drying for a Short Period Upon the Hatching of Inbred Eggs of Hydatina Senta.

Eggs Kept Wet.		Eggs Dried and Remoistened.	
Date.	Number Hatching.	Date.	Number Hatching.
Feb. 25 to Mar. 8	0	Feb. 24 to Mar. 4	0
Mar. 9	1	Mar. 5	1
Mar. 10 to 14	0	Mar. 6 to 14	0

Unlike the cross-fertilized eggs of Tables X. and XI., drying for a few hours neither increased the number of eggs that hatched nor hastened their time of hatching.

The foregoing experiment was repeated with inbred eggs of a line that regularly hatched more than half its eggs. The eggs remaining unhatched after five weeks were divided into two lots, one of which was dried over night and then remoistened. Though both were kept three weeks, not one egg in either lot hatched. Drying neither increased nor hastened the hatching.

Dried for Periods of Moderate Length.—These experiments differed from the foregoing in that all of the eggs were dried in one half of the experiment, instead of only those which failed to hatch under ordinary conditions. Inbred eggs were used, and drying occurred about the time when hatching was due to begin, that is, a week after the eggs were laid. Hatching began three or four days after the eggs were remoistened. Observations were continued for a month after remoistening. One lot of eggs was kept wet as a control, one was dried over night, one dried two weeks, and a fourth dried four weeks. The experiment was performed three times. The totals, without daily records, are given in Table XIII. The second division of this table really belongs to the preceding section of this paper, since it involves only a short period of drying, but it seems best to retain it here for the sake of comparison.

TABLE XIII.

Showing the Effect of Drying for Various Periods Upon the Viability of the Fertilized Eggs of Hydatina Senta. Inbred Eggs Were Used.

Experiment.	Eggs Kept Wet.		Dried Over Night.		Dried Two Weeks.		Dried Four Weeks.	
	Number of Eggs.	Number Hatching.	Number of Eggs.	Number Hatching.	Number of Eggs.	Number Hatching.	Number of Eggs.	Number Hatching.
A	75	40	78	35	76	18	72	0
B	55	26	41	6	50	12	53	0
C	39	23	33	19	38	6	27	0
Total.......	169	89	152	60	164	36	152	0
Percentage hatching ..	52.6		39.4		21.9		0.0	

There is plain indication in these results that drying, even for a short time, reduces the number of eggs that will hatch when again placed in water; and that the longer the period of desiccation, up to the limit of complete inhibition, the fewer the eggs that hatch.

Dried for a Long Period.—No experiment with control, involving a longer period of desiccation than four weeks, was performed; but that some eggs could withstand longer desiccation was shown. One lot of 344 cross-fertilized eggs and another of 100 inbred eggs, all from sources not used in the experiments described in this paper, were kept in dried condition from June 29 to March 23, or about nine months, when they were remoistened. In about three weeks thereafter three eggs of the first group and one of the second hatched.

All these eggs simply rested on the bottom of a watch glass when dried. It is not improbable that when the eggs, on drying, are supported by mud or sand they may remain desiccated longer and still hatch when remoistened. But even in mud the possible period of desiccation is not indefinitely long; for out of a lot of fertilized eggs in dried mud I have secured numerous young rotifers after three months of desiccation, but no eggs hatched from this lot after two years.

Summary and Discussion.

In former papers (Shull, 1913, 1915) the inheritance of size of parthenogenetic eggs, the time of development of parthenogenetic eggs, the place of laying parthenogenetic eggs (surface film or bottom), the viability of fertilized eggs (proportion that hatch), and the contractility of foot muscles was described. In this paper is shown to what extent these inherited characters may have been modified by such external agencies as temperature, oxygen, and desiccation.

It was found that if the water in which the rotifers live was exposed to an atmosphere containing more than the usual proportion of oxygen, a greater proportion of the eggs were laid at the bottom of the vessel. Under ordinary conditions the rotifers probably come to the surface because of the greater quantity of dissolved oxygen there. Those lines which normally lay their eggs mostly at the bottom probably either require less oxygen or get their oxygen more easily than lines which lay their eggs at the surface film. The hereditary character involved may therefore be the oxygen requirement or the permeability to oxygen.

If the rotifers were kept at a low temperature, their eggs were laid more largely at the bottom. This may be due to the greater concentration of oxygen in the water at low temperature, so that it is unnecessary to come to the surface so much; or to the low metabolism and hence low oxygen requirement of the animals; or to both of these reasons.

Excess of oxygen increased the size of the parthenogenetic eggs only very slightly, or not at all. Excess of oxygen decreased the time of egg development only very slightly, or not at all. It may have made the time of development a trifle more uniform, though this is hardly proven.

Rotifers kept in oxygenated water showed, when killed in Bouin's fluid, a trifle less contraction of the foot muscles than did other rotifers, though the difference was too small to constitute a proof of the action of oxygen.

Since the place of laying the eggs (surface or bottom) was so much more greatly affected by oxygen than were the other three characters tested, it would seem rather improbable even if the genetic results (Shull, 1915) had permitted such an assumption, that the several characters in which the English and Nebraska lines differ as described in the introduction to this paper, were really but a single character with several manifestations. The results described in this paper, therefore, harmonize with the genetic results previously obtained.

Freezing fertilized eggs that had remained unhatched for five to seven weeks did not induce any of them to hatch later. However, when eggs were kept from the time of laying at a low and variable temperature above freezing, more of them hatched than when kept at room temperature.

When fertilized eggs were kept in oxygenated water, a somewhat smaller proportion of them hatched than in untreated water.

Cross-fertilized eggs which had remained for seven weeks under ordinary conditions without hatching were dried over night and then remoistened. A considerable number of them were thereby caused to hatch. Inbred eggs, however, when tested in the same way, did not respond to drying. Even inbred eggs that were dried shortly after they were laid (not merely

those which failed to hatch otherwise) did not show any increase in the proportion of viable eggs. Indeed, drying had just the opposite effect on inbred eggs. Even when the eggs were kept dry only a few hours, the percentage of them that hatched was reduced; and the longer the eggs were kept dried the fewer of them hatched. Those that remained dry for four weeks did not hatch at all.

Other lines were not as sensitive to drying, for out of one lot of eggs that were dry for nine months, several eggs hatched when remoistened.

In view of the results of desiccation of inbred eggs, it is conceivable that the hatching of cross-fertilized eggs after drying was due merely to the hastening of the development of eggs whose hatching would otherwise have been spread over a long period. If we had for comparison only the experiments with inbred eggs which were dried immediately after laying, and those with cross-fertilized eggs that were dried after they had been allowed abundant time in which to hatch and had not done so, the conclusion just stated would seem not merely conceivable, but probable. However, since inbred eggs were also dried after their normal hatching period was past, and failed to hatch subsequently, whereas cross-fertilized eggs thus treated did hatch, I am inclined to believe that drying for a few hours actually caused some cross-fertilized eggs to hatch which would not have done so without drying. From the physiological viewpoint, such a difference between inbred eggs and cross-fertilized eggs is not at all improbable.

BIBLIOGRAPHY.

Shull, A. F.

'13 Inheritance in Hydatina senta. I. Viability of the resting egg and the sex ratio. Jour. Exp. Zoöl., Vol. 15, no. 1, July, pp. 49–89.

'15 Inheritance in Hydatina senta. II. Characters of females and their parthenogenetic eggs. Jour. Exp. Zoöl., Vol. 18, no. 1, January, pp. 145–186.